

Appln. No. 10/038,676
Amdmt. dated October 14, 2004
Reply to Office Action of August 11, 2004

Amendments to the Specification:

Please replace the paragraph on page 9, lines 6-14, with the following amended paragraph:

The compositions of the invention preferably have Bacillus spores present in sufficient concentrations to effect a viable spore count of between 10⁶ cfu to 10⁹ cfu per gram of dry composition. All preferred Bacillus spores herein have been deposited at the American Type Culture Collection ("ATCC"), 10801 University Blvd., Manassas, VA. 20110-2209, under accession numbers indicated herein. The strains of B. subtilis and B. licheniformis were deposited August 27, 2004, while the strains of B. laterosporous were deposited prior to August 27, 2004. Preferably, the Bacillus spores come from Bacillus selected from the group consisting of Bacillus laterosporus (ATCC _____) (ATCC PTA-3952), Bacillus laterosporus (ATCC _____) (ATCC PTA-3593), Bacillus licheniformis (ATCC _____) (ATCC PTA 6175), Bacillus subtilis (ATCC _____) (ATCC PTA-6174), and mixtures thereof. More preferably, the Bacillus spores come from mixtures of two or more of Bacillus laterosporus (ATCC _____) (ATCC PTA-3952), Bacillus laterosporus (ATCC _____) (ATCC PTA-3593), Bacillus licheniformis (ATCC _____) (ATCC PTA 6175), and Bacillus subtilis (ATCC _____) (ATCC PTA-6174).

Please replace the paragraph on page 13, lines 3-21, with the following amended paragraph:

Dry ingredients, including decontaminated manure (ranging from about 20 to about 70 weight percent of the solid product formulations), humic acids (preferably ranging from about 5 to about 25 weight percent of the solid product formulations) and N-P-K compounds (preferably ranging from about 20 to about 60 weight percent of the solid product formulation, if used) are ground to mesh size ranging from about 50 to

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about 400 mesh, (_____ cm to about _____ cm) more preferably ranging from about 100 to about 150 mesh (_____ cm to about _____ cm). These ingredients are blended (in no specific order), and then conveyed into a granulator where an aqueous spore suspension of the *Bacillus* microorganisms is sprayed onto the revolving dry ingredients. Preferably, from about 5 to about 15 percent of dechlorinated water is applied by weight, said water containing all of the *Bacillus* spores required to produce the batch. The moistened ingredients are then conveyed into a rotating drying tunnel/drum where the temperature of the product is preferably maintained at temperature ranging from about 70 to about 90°C for a time ranging from about 1 to about 30 minutes, more preferably from about 5 to about 15 minutes, subsequently, the product enters a cooling tunnel and is cooled rapidly to a temperature ranging from about 30 to about 40°C. The resulting prills, formed by the rotation and drying process, are preferably screened for size and filled into fertilizer bags, completing the process.

Please replace the paragraph on page 15, lines 15-23, with the following amended paragraph:

In preferred embodiments of the present invention, strains of *Bacillus* species that are capable of exerting a positive effect on the microbial composition of the rhizosphere are utilized. In particular, it is advantageous to use strains that produce significant increases in the populations of Actinomycetes and nitrogen-fixing bacteria within the rhizosphere. Experiments #1 and #2 demonstrate this effect in carrot rhizosphere soil. In Experiment #1, a strain of *Bacillus laterosporus* (ATCC _____) (ATCC PTA-3952) causes an increase of > 1 log in Actinomycetes and nitrogen-fixing bacteria. In Experiment #2, a strain of *Bacillus licheniformis* (ATCC _____) (ATCC PTA 6175) produces a similar result.

Please replace the paragraph bridging pages 15 and 16 with the following amended paragraph:

One of the critical discoveries of the present invention involves the unique application of animal manure in potentiating the effect of the Bacillus microorganisms; specifically, chicken or swine manure, produced without litter or bedding, and produced from animals not receiving growth-promoting antibiotics in their feed. Chicken manure, for example, contains the following amounts of N, P (P_2O_5), and K (K_2O) in lbs. per 1,000 U.S. gallons: N = 80, P_2O_5 = 36, K_2O = 96. In addition, there are many organic compounds that may serve as microbial nutrients. Fresh layer chicken manure, 13 % dry matter content, contains over one billion or 1×10^9 cfu/gram of aerobic/facultative microorganisms, drying at 65°C reduces this count, slightly, by approximately one-half. Such manure, nutrient content notwithstanding, cannot be used in the present invention. Manure with high concentrations of microorganisms will grossly contaminate the fertilizer formulations of this invention and result in poor growth of probiotic, Bacillus microorganisms in the rhizosphere. Experiment #3 illustrates this point – *B. laterosporus* (ATCC _____) (ATCC PTA-3593) grown in sterile 1% chicken manure grows out to 1×10^8 cfu/ml, proving that manure ingredients support the growth of Bacillus; however, in 1% raw chicken manure, *B. laterosporus* (ATCC _____) (ATCC PTA-3593) develops a count below 1×10^5 cfu/ml. The present invention requires substantially dry manure, moisture content preferably less than 20 weight percent, preferably less than 15 weight percent, chicken or swine origin, that has a microbial plate count below ten million or 1×10^7 cfu/gram (aerobic/facultative: total plate count on tryptic soy agar, 3 days, 32°C), preferably below one million or 1×10^6 cfu/gram. This represents a 100 to 1,000 fold reduction, two-three logs, compared to the total count in fresh manure. When manure with a microbial content below one million cfu/gram is used according to the teachings of the present invention, the resulting fertilizer formulations preferably have a Bacillus purity of 90 percent or greater. For lack of definitive terminology this inventor will use the term “decontaminated manure” for manure that has a reduced viable plate count according to the specifications stated above.

Please replace the paragraph on page 17, lines 15-30 with the following amended paragraph:

A second, unexpected discovery of the present invention relates to the stabilizing effect that dry, decontaminated manure has on the viability of Bacillus spores to storage under adverse conditions (35 C at > 80% relative humidity). This is clearly indicated in Experiment #6 where the effect of blending spores of *B. laterosporus* (~~ATCC _____~~) (ATCC PTA-3952), *B. licheniformis* (~~ATCC _____~~) (ATCC PTA-6175), or *B. subtilis* (~~ATCC _____~~) (ATCC PTA-6174) in decontaminated chicken or swine manure is compared to a blend prepared in a dry N-P-K mixture and a blend containing manure and N-P-K. After 6 months at 35 C/80% relative humidity, blends containing decontaminated manure had spore counts significantly higher (> one log) compared to the N-P-K mixture. This is relevant to the shelf-life of fertilizer formulations containing N-P-K ingredients that are prepared according to the teachings of the present invention. I do not understand the mechanism of this effect, it may relate to a protective effect rendered by certain complex organic compounds present in the treated manure. It is well known in the trade that sterile soil is a protective medium for Bacillus spores, manure decontaminated to the extent specified by the present invention may duplicate the effect of soil to some degree or, some heretofore unknown effect may be operating.

Please replace the paragraph bridging pages 19 and 20 with the following amended paragraph:

B. laterosporus (~~ATCC _____~~) (ATCC PTA-3593), 5×10^8 spores/ml, was applied to one acre of carrots at planting, emergence, and one week post emergence at 1.5 liters per application. Applications were made via drip irrigation, sandy-loam soil, southern California location. A control plot was treated with water only. The same level of N-P-K fertilizer was applied to both plots. At two weeks post emergence twelve samples of rhizosphere soil were taken from each plot and co-mingled aseptically. The co-mingled samples were held at 5 C and analyzed within 24 hours for Actinomycetes, nitrogen-fixing bacteria, and gram positive/gram negative ratio. Note: Bacillus bacteria are gram positive. Results are listed in Table 2. This data provides evidence that *B.*

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laterosporus (ATCC _____) (ATCC PTA-3593) stimulates the growth of beneficial microorganisms in the rhizosphere.

Please replace the paragraph on page 20, lines 25-35 with the following amended paragraph:

B. licheniformis (ATCC _____) (ATCC PTA-6175), 4×10^8 spores/ml, was applied to one acre of carrots at planting, emergence, and one week post emergence at 1.5 liters per application. Applications were made via drip irrigation, sandy-loam soil, southern California location. A control plot was treated with water only. The same level of N-P-K fertilizer was applied to both plots. At two weeks post emergence twelve samples of rhizosphere soil were taken from each plot and co-mingled aseptically. The co-mingled samples were held at 5 C and analyzed within 24 hours for Actinomycetes, nitrogen-fixing bacteria, and gram positive/gram negative ratio. Note: Bacillus bacteria are gram positive. The results are presented in Table 3. This data provides evidence that *B. licheniformis* (ATCC _____) (ATCC PTA-6175) stimulates the growth of beneficial microorganisms in the rhizosphere.

Please replace the paragraph on page 21, lines 12-26 with the following amended paragraph:

B. laterosporus (ATCC _____) (ATCC PTA-3952) and *B. licheniformis* (ATCC _____) (ATCC PTA-6175) were tested for growth in 1% aqueous solutions of layer chicken manure: sterilized, decontaminated, and raw manure solutions were evaluated. Decontaminated manure was treated for 3 years in a compost pit and then sun dried for three weeks, it had a total microbial plate count of $< 10^6$ cfu/gm. The raw manure used had a total microbial plate count of 3×10^9 cfu/gram. Bacillus cultures (5×10^{18} spores/gm) were inoculated into the manure solutions at 1% by volume and subsequently incubated at 34 C for 48 hours; this was done in 250 ml baffled Erlenmeyer flasks shaken constantly at 125 rpm. Total Bacillus plate counts and total

aerobic/facultative counts were made after 48 hours using tryptic soy agar incubated at 32 C for 72 hours. The Bacillus counts were made by first heating the sample for 10 minutes at 80 C to kill non-spore forming bacteria and were then incubated aerobically to recover only Bacillus. Results are listed in Table 4. This data provides evidence that Bacilli grow well in chicken manure if it is sterilized or decontaminated but do not grow well in raw manure due to its high concentration of microbial contaminants.

Please replace the paragraph bridging pages 22 and 23 with the following amended paragraph:

One acre plots of Chip variety potatoes, planted in central Utah, were utilized for this experiment. One plot was treated with a Bacillus spore suspension comprised of 4 x 10⁸ cfu/ml of *B. laterosporus* (~~ATCC _____~~) (ATCC PTA-3952) and 1 x 10⁸ cfu/ml of *B. licheniformis* (~~ATCC _____~~) (ATCC PTA-6175); one liter of this solution was applied via a sprinkler irrigation system at planting, at emergence, and at one and three weeks post emergence. Another plot was treated with 20 gallons of hypochlorite decontaminated layer chicken manure (1% of 68% active calcium hypochlorite into 30% slurry of manure, reacted for 24 hours at 25 C) added in four divided applications as for the Bacillus cultures. Another plot was treated with both the Bacillus spore suspension and the decontaminated chicken manure, same levels as indicated above. Finally, two plots were maintained as controls. Control plots received water only. The base fertilizer program used for all treatment and control plots was: 800 lbs 16-20-0-15 and 375 lbs of 21-0-0-24 (these represent percentages of N-P-K-S with P and K calculated as P2O5 and K2O, respectively). The yields were measured at harvest and reported as # of 100 lb (CWT) sacks per acre – four replications were made per plot and the averaged results are reported below. The data are listed in Table 5, which are average of replicates. This data is evidence that a synergistic response – maximum yield of 529 x 100 lb sacks/acre – is realized when the Bacillus spore suspension is used in combination with decontaminated manure.

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Please replace the paragraph on page 26, lines 15-18 with the following amended paragraph:

The following *Bacillus* cultures were examined:

- B. laterosporus (ATCC _____) (ATCC PTA-3952) code below = B. lat.
- B. licheniformis (ATCC _____) (ATCC PTA-6175) code below = B. lich.
- B. subtilis (ATCC _____) (ATCC PTA-6174) code below = B. sub.

Please replace the paragraph bridging pages 34 and 35 with the following amended paragraph:

Separate aerobic fermentations were carried on two probiotic species of *Bacillus* bacteria, each species was grown separately. *Bacillus laterosporus* (ATCC _____) (ATCC PTA-3952) and *Bacillus licheniformis* (ATCC _____) (ATCC PTA-6175), strains that enhance the numbers of beneficial microorganisms in the rhizosphere (see Experiments #1 & 2.), were grown in sterile tryptic soy broth (TSB) in 1,000 liter aerobic fermentors for 48 hours at 32 C while continually agitated at 150 rpm and continually sparged with sterile air at 1,000 liters/minute. At 48 hours the pH was adjusted to 7.0 with either NaOH or HCl and the liquid spore suspensions were cooled to 20 C and held until used. For the purpose of the present example, a blend was prepared from the two spore suspensions: 80% by volume of *B. laterosporus* was blended with 20% by volume of *B. licheniformis* and the resulting spore count of the blend was determined to be 8 x 10⁸ cfu/ml (1 ml = 1 gram). This blend was used to inoculate the formulation of the present example during the granulation step described below.

Please replace the paragraph on page 37, lines 7-18 with the following amended paragraph:

An aerobic fermentation was carried on two probiotic strains of *Bacillus*, the strains were grown together in the fermentor but maintained separately in shake flasks which served as the inoculum for the fermentor. *Bacillus laterosporus* (ATCC _____) (ATCC PTA-3952) and *Bacillus laterosporus* (ATCC _____) (ATCC PTA-3593), a species that enhances the numbers of beneficial microorganisms in the rhizosphere (see Experiments #1 & 2.), were grown in sterile tryptic soy broth (TSB) in 1,000 liter aerobic fermentors for 48 hours at 32 C while continually agitated at 150 rpm and continually sparged with sterile air at 1,000 liters/minute. At 48 hours the pH was adjusted to 7.0 with either NaOH or HCl and the liquid spore suspensions were cooled to 20 C and held until used. The resulting spore count of this two strain culture was determined to be 5 x 10⁸ cfu/ml (1 ml = 1 gram). This blend was used to inoculate the formulation of the present example during the granulation step described below.

Please replace the paragraph on page 39, lines 14-25 with the following amended paragraph:

An aerobic fermentation was carried on a probiotic strain of *Bacillus subtilis* (ATCC _____) (ATCC PTA-6174). The strain was grown in a 1,000 liter fermentor inoculated from a TSB shake flask culture, multiple shake flasks served as the inoculum which was added at 1% of the working volume of the fermentor. This is a strain of *Bacillus subtilis* that has proven to be antagonistic to plant pathogenic fungi. The strain was grown in sterile tryptic soy broth (TSB) in the fermentor for 48 hours at 32 C while under agitation agitation at 150 rpm and sparging with sterile air at 1,000 liters/minute. At 48 hours the pH was adjusted to 7.0 with either NaOH or HCl and the liquid spore suspension was cooled to 20 C and held until used. The resulting spore count of this culture was determined to be 7 x 10⁸ cfu/ml (1 ml = 1 gram). This blend was used to inoculate the formulation of the present example during the granulation step described below.